THE UNIVERSITY OF ALBERTA

PARASITES OF RED SQUIRRELS, TAMIASCIURUS HUDSONICUS, IN ALBERTA

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Parasites of Red Squirrels, Tamiasciurus hudsonicus, in Alberta", submitted by Soo-Jeet Chai in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

A total of 163 red squirrels, Tamiasciurus hudsonicus (Erxleben, 1774), were examined for parasites (81 from Gorge Creek; 82 from Rochester, Alberta). The ectoparasites found on red squirrels from the Gorge Creek area were Orchopeas caedens and Monopsyllus vison (Siphonaptera), Hoplopleura sciuricola (Anoplura), Ixodes sp. (Acarina), and mites (unidentified). Six cestode, four nematode and three protozoan species were found. Andrya primordialis and Hymenolepis horrida were found in the small intestine and cysticerci of Taenia rileyi, T. mustelae and Cladotaenia sp. were encysted in the liver. Plerocercoids of Paruterina candelabraria were recovered from the liver and mesentric lymph nodes of one red squirrel. Cysticerci of Cladotaenia sp. and plerocercoids of P. candelabraria had not been reported previously in red squirrels. Nematodes were found in three locations of the alimentary tract: Physaloptera sp. in the stomach, Citellinema bifurcatum and Ascaris sp. in the small intestine, and Syphacia sp. in the cecum.

Gametocytes of Hepatozoon sp. were found in the blood smears. Oocysts of Eimeria tamiasciuri and E. toddi were detected in the fecal material. E. toddi had not been reported previously from Canada.

The endogenous cycle of E. tamiasciuri consisted of two asexual generations and gametogony. The endogenous stages were found only in the epithelial cells of the small

intestine. The first generation schizonts infected the epithelial cells of the crypt of Lieberkühn. Developing schizonts were found 24, 48, 72 and 96 hours after inoculation. Mature first generation schizonts were found 72 and 96 hours after inoculation. Second generation schizonts, which were smaller and produced fewer merozoites, were found in the epithelial cells of the villi 72 hours after inoculation. Developing gametocytes were found at the same site as the second generation schizonts. Gametocytes were present as early as 72 hours after inoculation. Oocysts were detected in the feces of one experimentally infected squirrel at 5½ days; thus, the prepatent period can be tentatively assumed to occur within that time.

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I INTRODUCTION

1. Distribution and reported parasites of *Tamiasciurus hudsonicus*.

Tamiasciurus hudsonicus, commonly known as the North American red squirrel, occupies a wide geographic range, extending from Alaska and northern Quebec southward, in the Rockies to southern New Mexico and, in the Appalachians to South Carolina. Within this range it is found in the hardwood forests of eastern North America, the coniferous forests of the west and north, and the dwarf conifers of the far north.

There have been only a few reports on the parasites of red squirrels in Canada. Rausch and Tiner (1948) found *Citellinema bifurcatum* (Nematoda) in red squirrels in Manitoba. Holland (1949) summarized the fleas found in Canada in the monograph "Siphonaptera of Canada". Gabbutt (1961) found one specimen of *Orchopeas caedens* from *T. hudsonicus* in central Labrador. From 1960 to 1963, a survey on the parasites of red squirrels in Alberta was conducted by Holmes (unpublished). Seventy-one squirrels were examined from the Gorge Creek area in Alberta. The parasites found included fleas, lice, mites, nematodes, cestodes and larval cestodes. Some of the fleas were *Tarsopsyllus coloradensis* and the larval cestodes were *Taenia rileyi* and *T. mustelae*. Fleas other than *T. coloradensis* were also found, but they were

not identified. Mahrt (pers. comm.) found Eimeria tamiasciuri in five red squirrels in Alberta during the summer of 1967. Recently Soon and Dorney (1969) reported the occurrence of E. tamiasciuri from T. hudsonicus in Ontario.

Outside Canada the earliest report on parasites of red squirrels was made by Douthitt (1915), in which he found Andrya primordialis (Cestoda) from T. hudsonicus in Colorado. Green (1938) found fleas (Orchopeas wickhami, Epithedia wenmanni), mites (Euhaemogamasus sp., Atricholaelaps sp.) and lice (Neohaematopinus sciurinus) on red squirrels in Minnesota. Rausch and Tiner (1948) studied the helminths of red squirrels from the North Central States and reported Fibricola nana (Trematoda) and the following nematodes: Rictularia sp., Ascaris sp., Citellinema bifurcatum, Heligmodendrium hassalli, Strongyloides sp., and Syphacia thompsoni. Botfly larvae (Cuterebra emasculator) were found in red squirrels in northern Wisconsin (Dorney, 1965). Kim (1966) described and illustrated 20 species of Enderleinellus (lice), which infect squirrels. Among them a new species, E. tamiasciuri, was described from T. hudsonicus. Dorney (1967) found lewisi-like trypanosomes in blood smears from red squirrels in Wisconsin. Lichtenfels and Haley (1968) studied the parasites of red squirrels in Maryland. They found Strongyloides robustus, Capillaria americana, Citellinema bifurcatum and Syphacia thompsoni. This was a new host record for the first two nematodes.

The coccidium, Eimeria tamiasciuri (Protozoa, Emeriidae), was described by Levine, Ivens and Kruidenier (1957) from a single red squirrel from Arizona. Bullock (1959) found it in two T. hudsonicus loquax in New Hampshire, and Dorney (1962) found the same species in 98% of 50 T. hudsonicus in Wisconsin. Soon and Dorney (1969) found it in 92.3% of 13 T. hudsonicus in Ontario. A second coccidium, E. toddi, was found in 6% of 36 red squirrels in northeastern Wisconsin (Dorney, 1962). The endogenous stages of both E. tamiasciuri and E. toddi are unknown.

2. Generalized life cycle of Eimeria.

Eimeria has a rather complex life cycle consisting of three phases: sporogony, schizogony and gametogony. Sporogony occurs after oocysts are discharged with fecal material from the animal. An unsporulated oocyst develops in the presence of moisture and oxygen into the infective stage. This sporulation process (sporogony) results in the formation of eight infective bodies (sporozoites) in each oocyst.

When sporulated oocysts are ingested the sporozoites are liberated into the lumen of the intestine by a process called excystation. Sporozoites penetrate into the intestinal epithelium and enter into the second phase of the life cycle, schizogony, which is a type of asexual reproduction. Schizonts develop and merozoites are formed. These merozoites are liberated from the schizont and then infect new host cells,

thus beginning another asexual generation. The number of asexual generations vary depending upon the species of Eimeria.

Gametogony (sexual reproduction) begins after the final generation of merozoites have penetrated new host cells. Some of the merozoites develop into macrogametes while others into microgametocytes. The macrogamete does not undergo nuclear division, but grows in size as it develops. The nucleus of the microgametocyte divides many times producing numerous microgametes. Following fertilization by a microgamete, the macrogamete develops into an unsporulated oocyst with the formation of the oocyst wall. The oocyst ruptures from its host cell and is discharged from the host in the feces.

3. Research objectives.

The objectives of my research were to conduct a survey of the parasites of red squirrels in Alberta, and to describe the endogenous stages of the coccidium, Eimeria tamiasciuri.

There are several reasons for this study. The initial investigation is designed primarily to determine the parasitic fauna of a specific wild animal or group of related hosts. This information can then serve as a basis for further host-parasite studies. A survey as outlined in the objectives above also serves well to train the graduate student to become proficient in the identification of a variety of para-

sites. Knowledge of the endogenous stages of Eimeria tamiasciuri will be valuable since an investigator must know the life cycle of the parasite as background information for future studies.

II MATERIALS AND METHODS

1. Survey of parasites.

Red squirrels were collected by shooting and live-trapping in the spruce forests within four miles of the R. B. Miller Biological Station, Gorge Creek, Alberta. Sixty-three squirrels were shot with a .410 gauge shotgun during May to August, 1968; eight squirrels were live-trapped during this period. An additional 18 red squirrels were shot and 16 more were trapped in May, 1969. Only four of these 16 squirrels survived confinement in cages. In addition, frozen viscera from 82 red squirrels collected during March to August, 1968 by personnel at a biological station of the University of Wisconsin, Rochester, Alberta, were analyzed.

Squirrels that were shot were placed in plastic bags immediately to prevent the ectoparasites from escaping. After returning to the laboratory the hosts were examined for ectoparasites. A skin dissolving technique (Hopkins, 1949) was occasionally used for more thorough recovery of ectoparasites. Ectoparasites were preserved in 70% ethyl alcohol. Representative specimens of fleas, lice and mites were cleared in 10% KOH, dehydrated in 90, 95, and 100% ethyl alcohol, cleared in methyl salicylate and mounted in balsam. Ticks were studied unmounted.

Measurements of length of body, tail and hind foot of squirrels were recorded in mm. and body weight in gm. prior

to necropsy (see appendix). Thin blood smears were stained with Giemsa. Each blood smear was examined microscopically at 400X for ten minutes. Tapeworms were preserved in alcohol-formalin-acetic acid (AFA). They were then stained with Ehrlich hematoxylin and permanent slides were prepared for identification. Larval cestodes encysted in the liver were recovered and a hook squash of each was prepared by mounting the rostellum of the larva directly in permount. Morphology of hooks was used as criteria for identification. Nematodes were fixed and stored in 70% ethyl alcohol with 5% glycerol. They were cleared in lactophenol before identification. Feces were collected from the rectum and examined by the centrifugal coverslip floatation technique (Levine, 1961) for coccidial oocysts and helminth eggs.

Several references were used as aids in identification of parasites: "The Siphonaptera of Canada" (Holland, 1949) for fleas; "The Ixodoidea of Canada" (Gregson, 1956) for ticks; and "Systema Helminthum", Volumes II and III (Yamaguti, 1959, 1961) for cestodes and nematodes, respectively. "The Coccidian Parasites (Protozoa, Sporozoa) of Rodents" (Levine and Ivens, 1965) was used for the identification of coccidia.

2. Studies on the coccidium, Eimeria tamiasciuri.

Twelve live-trapped squirrels were kept in wire cages with mesh floors. They were fed Purina laboratory chow for rats. All 12 squirrels had natural infections of E. tamiasciuri. Fresh feces were collected, mixed with a 2.5% potas-

sium dichromate solution, poured into petri dishes to a depth of 5 mm. or less, and incubated at room temperature. After sporulation the oocysts were stored at 3 - 5° C until needed for experimental infection.

a) Prepatent period

Two of the 12 squirrels were used to study the prepatent period of E. tamiasciuri. Both squirrels were treated with a coccidiostat, sodium methiozine, to remove the endogenous stages of the coccidium prior to experimental inoculation. One squirrel was inoculated with sporulated oocysts of E. tamiasciuri by means of a rubber stomach tube. The second squirrel served as an uninoculated control. Feces were collected daily from both animals and examined for oocysts. The interval between the time of inoculation and the first appearance of oocysts in the feces, i.e. the prepatent period, was recorded.

b) Endogenous stages

Natural infections of E. tamiasciuri were removed from the squirrels by administration of a coccidiostat as described above. Each of four coccidia-free red squirrels was inoculated with 100,000 sporulated oocysts. They were killed at 24, 48, 72 and 96 hours after inoculation. Two additional squirrels were each inoculated with 100,000 oocysts, and subsequently killed at 48 and 72 hours. The intestinal tract was removed and flushed with Zenker's solution. Tissue at 5 cm. intervals along the intestine was fixed in Zenker's solution for 6 - 8 hours. Sections cut at 7 μ were stained with either Harris'

hematoxylin and eosin Y, or Harris' hematoxylin alone.

Histological sections were also prepared as described above from two squirrels which were naturally infected with E. tamiasciuri.

Free sporozoites were obtained for observation by in vitro excystation of oocysts using the method described by Doran and Farr (1962), except that oocysts were ruptured with a ground-glass tissue grinder and 2% bovine bile was used.

Observations and measurements of oocysts, sporocysts, and the endogenous stages were made with a Leitz Laborlux microscope equipped with fluorite objectives.

III RESULTS

1. Ectoparasites

The following arthropods were found on red squirrels from the Gorge Creek area: both Monopsyllus vison and Orchopeas caedens (81 of 81, 100%); Ixodes sp. (6 of 81, 7%); Hoplopleura sciuricola (5 of 81, 6%); and unidentified mites (5 of 81, 6%). Intensities of ectoparasites per host ranged from two to twenty for fleas, one to three for ticks, one to over one hundred for lice, and one to two for mites.

2. Helminths

a) Cestoda

Adult Hymenolepis horrida and Andrya primordialis were recovered from the small intestine. Cysticerci were found encysted in the liver of 13.5% (22 of 163) of the squirrels examined. They were identified as Taenia rileyi, T. mustelae and Cladotaenia sp. Intensities were one to two for H. horrida, one to four for A. primordialis, one to seven for T. rileyi, and two to 31 for Cladotaenia sp. Two specimens of T. mustelae were recovered from a single squirrel. In one red squirrel 16 plerocercoids of Paruterina candelabraria were found encysted in the liver and the mesentric lymph nodes. A comparison of parasites of red squirrels from Gorge Creek and Rochester is given in Table 1.

Monthly prevalence of cestodes of red squirrels from

Table 1. Prevalence of helminths of *Tamiasciurus hudsonicus* from Gorge Creek (1968-69) and Rochester, Alberta (1968).

	Gorge Creek (81 examined)	Rochester (82 examined)
	No. and percent of squirrels infected	No. and percent of squirrels infected
Cestodes:	28 (34.6%)	13 (15.8%)
*Taenia rileyi**	10 (12.4%)	10 (10.9%)
*Taenia mustelae**	1 (1.2%)	0
Cladotaenia sp.*	2 (2.4%)	0
*Paruterina candelabraria**	0	1 (1.2%)
Andrya primordialis	21 (25.9%)	4 (4.8%)
Hymenolepis horrida	5 (6.1%)	0
Nematodes:	20 (24.7%)	4 (4.8%)
Citellinema bifurcatum	11 (13.6%)	3 (3.6%)
Syphacia sp.	7 (8.7%)	1 (1.2%)
Ascaris sp.	0	1 (1.2%)
Physaloptera sp.	2 (2.4%)	0

* Larval stages of these cestodes.

Table 2. Monthly prevalence of helminths of T. hudsonicus from Gorge Creek (1968-69).

	1968				1969
Helminth	May (7)*	June (15)	July (32)	August (9)	May (18)
Taenia rileyi	0	2(13)**	4(12)	2(22)	2(11)
Taenia mustelae	0	0	1(3)	0	0
Cladotaenia sp.	0	0	0	0	2(11)
Andrya primordialis	1(14)	2(13)	10(31)	2(22)	6(33)
Hymenolepis horrida	0	0	5(16)	0	0
Citellinema bifurcatum	1(14)	2(13)	4(12)	1(11)	3(17)
Physaloptera sp.	0	0	1(3)	0	1(6)
Syphacia sp.	1(14)	0	1(3)	2(22)	3(17)

* Number of squirrels examined.

** Number and (percent) infected.

Table 3. Monthly prevalence of helminths of *T. hudsonicus* from Rochester (1968).

	1968					
Helminth	Mar. (12)*	Apr. (31)	May (13)	June (10)	July (5)	August (11)
Taenia rileyi	2(17)**	0	3(23)	2(20)	0	3(27)
Paruterina candelabraria	0	0	0	0	0	1(9)
Andrya primordialis	0	0	0	0	0	4(36)
Citellinema bifurcatum	0	0	1(8)	0	0	2(18)
Syphacia sp.	0	0	0	0	0	1(9)
Ascaris sp.	0	1(3)	0	0	0	0

* Number of squirrels examined.

** Number and (percent) infected.

Gorge Creek and Rochester are summarized (Tables 2, 3). Five cestode species were found in red squirrels from Gorge Creek area and three in those from Rochester. Taenia rileyi were found quite consistently throughout the summer in both locations. Andrya primordialis were found in red squirrels from Gorge Creek area and showed an increase in prevalence in July and August. In contrast, A. primordialis infected only red squirrels collected in August in Rochester. T. mustelae, Cladotaenia sp. and Hymenolepis horrida were not found in squirrels from Rochester whereas Paruterina candelabraria was not found in squirrels from the Gorge Creek area.

b) Nematoda

Nematodes were found in three locations of the alimentary tract: Physaloptera sp. was found in the stomach, Citellinema bifurcatum in the small intestine, and Syphacia sp. in the cecum. Intensities of nematodes ranged from one to twelve for Physaloptera sp., one to ten for Citellinema bifurcatum, and one to over 200 for Syphacia sp. In one red squirrel an immature specimen of Ascaris sp. was recovered from the small intestine. A comparison of Gorge Creek and Rochester data is given in Table 1.

Monthly prevalence of nematodes of red squirrels from Gorge Creek and Rochester are summarized (Tables 2, 3). Citellinema bifurcatum and Syphacia sp. were found throughout the summer in red squirrels from the Gorge Creek area. They were rare in squirrels from Rochester. Physaloptera

sp. was not found in red squirrels from Rochester. In contrast Ascaris sp. was not found in squirrels from Gorge Creek.

The frequency of helminth infection of red squirrels was 51% (41 of 81) for the Gorge Creek area and 18% (15 of 81) for Rochester. Five species of helminths were found in squirrels collected in August in Rochester. Only one or two species were found in the other months, except July when no parasites were found (Table 3).

3. Protozoa

Gametocytes of Hepatozoon sp. were found scattered between the blood cells in 28% (18 of 63) of the blood smears. They were elongate bodies with rounded ends. The nucleus was situated at the center of the cell. The cells ranged from 8 - 11 μ in length and 3 - 5 μ in width.

Two species of coccidia were found. Oocysts of Eimeria tamiasciuri were present in 97% (166 of 171) of the fecal samples. E. toddi oocysts occurred in 6% (10 of 171) of the squirrels. Fifty unsporulated E. toddi oocysts measured 29.1-46.5 X 24.5-33.7 μ (mean, 36 X 29 μ). Details of E. tamiasciuri oocysts are given below.

4. Studies on the coccidium, Eimeria tamiasciuri.

a) Prepatent period

The length of the prepatent period was determined from only one infected red squirrel. Oocysts appeared in the feces 5½ days after inoculation. The uninoculated control squirrel

remained negative during this period.

b) Exogenous stage

The oocysts of E. tamiasciuri were elongate ellipsoidal. The oocyst wall was smooth, colorless and composed of a single layer 1.5 μ thick. There was no micropyle or oocyst residuum. Sixty sporulated oocysts (Fig. 1) measured 18.6-33.5 X 11.2-18.6 μ, with a mean of 25.6 X 14.2 μ. The length/width ratio ranged from 1.3 - 2.2, with a mean of 1.55. Sporocysts were elongate ovoid with a prominent Stieda body and contained a dense granular residual material. A sporozoite, present at each end of the sporocyst, curled around the residuum. Excysted living sporozoites were sausage-shaped with a fine granular cytoplasm (Fig. 2). The anterior and posterior refractile bodies appeared as spherical clear areas, and were located near the nucleus. The nucleus was centrally positioned; however, detailed morphology was not seen with bright-field microscopy. Twelve sporozoites measured 7.5-12.3 X 3-4 μ. Freshly excysted sporozoites were motile, undergoing flexing and gliding movements.

c) Endogenous stages

Location: The endogenous stages of E. tamiasciuri were found in the small intestine. No parasites were seen in the cecum or colon. The maximum concentration of stages occurred in the jejunum. Endogenous stages developed only in the mucosal epithelium.

Schizogony: Two asexual generations were present. They were readily distinguished by their sites of infection,

size of schizonts, and the number of merozoites.

Young developing first generation schizonts (Fig. 3) were found 24, 48, 72 and 96 hours after inoculation. They occurred above the nuclei of epithelial cells in the crypt of Lieberkühn. Young schizonts were nearly round, and were surrounded by a vacuole in the stained preparations. A prominent refractile body was present in all young schizonts. This darkly stained spherical body about 2 μ in diameter was positioned eccentrically in the homogenous eosinophilic cytoplasm of the parasite. Nuclear division had already occurred in most schizonts since two to four nuclei were most commonly found. Nuclei appeared as dark dots scattered randomly in the cytoplasm. The schizonts were 3 - 9 μ in diameter. The host cell nucleus was slightly indented next to the side of the growing schizont.

Mature first generation schizonts (Fig. 4) were found 72 and 96 hours after inoculation. Mature schizonts were nearly spherical and contained 30 to 60 merozoites. Five schizonts measured 17.5 to 22 μ in diameter. The host cell was distended by the presence of the schizont. This enlargement also caused distortion of neighboring cells. The host cell nucleus was crescent in shape and was pushed downwards towards the base of the parasitized cell. The merozoites were spindle-shaped, with each having one faintly stained nucleus. Merozoites measured 8.6-12.5 X 1-1.2 μ (Fig. 5).

Second generation schizonts were found 72 hours after inoculation. Gametocytes were also present at this time,

indicating that second generation schizonts had occurred earlier than 72 hours; however, these were not observed. Schizonts were also seen in sections from the two squirrels which had natural infections. Second generation schizonts (Figs. 6, 7) infected epithelial cells of the villi only; none were found in the crypt of Lieberkühn. Mature second generation schizonts were much smaller than the first generation schizont (Figs. 4, 7). The average size of ten mature second generation schizonts was 6.2 X 5 μ. Each second generation schizont contained four to sixteen merozoites, depending on the sections. In one schizont that had been sectioned transversely, 16 merozoites could be seen clearly. In sections that were cut longitudinally, the average merozoite measured 6.0 X 1.2 μ.

Gametogony: Sexual stages were seen in the epithelial cells of the intestinal villi 72 hours after inoculation. Numerous sexual stages (gamonts) were also present in the tissue sections of the two squirrels with natural infections. The macrogamete to microgametocyte ratio was ten to one.

Microgametocytes could be readily distinguished from macrogametes by the presence of numerous nuclei (Fig. 8) as opposed to the single large nucleus and nucleolus of each macrogamete (Figs. 12, 13). In addition, the cytoplasm of young microgametocytes was less granular and basophilic than young macrogametes (Figs. 8, 12). Microgametocytes were not seen in the uninucleate stage. The nuclei were scattered randomly throughout the cytoplasm of the microgametocyte

(Fig. 8) in the early stages of nuclear division. The next stage of development and probably the final stage of nuclear division was characterized by chromatin massing along one side of each nucleus. This gave the nucleus a C-shape appearance (Fig. 9). The chromatin then condensed into a darkly stained spherical mass (Fig. 10). These spherical nuclei then moved to a peripheral position within the microgametocyte, and the remaining cytoplasm formed a prominent residual mass in the center (Fig. 10). The final development of the microgametes from the spherical nuclear stage into mature microgametes (Fig. 11) was not observed. Flagella of individual microgametes were not observed. Mature microgametocytes contained 50 to 60 microgametes (Fig. 11). Six mature microgametocytes measured 8.6-14.9 X 7.4-11.0 μ (mean, 11.0 X 8.7 μ). The microgametocyte appeared to have no effect on the host cell apart from causing distortion to its nucleus.

Macrogametes were characterized by having a large distinct nucleus throughout development (Fig. 12). Young macrogametes were recognizable by their broadly ellipsoidal shape, basophilic cytoplasm, and large nuclei each with a prominent nucleolus. Ten young macrogametes were 4.9-6.1 X 3.7-4.9 μ (mean, 5.5 X 4.4 μ). As the macrogametes developed the cytoplasm became more granular (Fig. 13). Two types of granules were seen in maturing macrogametes (Figs. 14, 15). The larger basophilic granules moved from the interior to the periphery (Fig. 15) to later form the oocyst wall. The second type of granules were eosinophilic and remained in the cytoplasm

along with the vacuoles. This gave the macrogamete a vacuolated appearance (Fig. 15). Seven nearly mature macrogametes averaged 10 X 6.2 μ. The host cell nucleus was pushed towards the base of the cell, and became indented where it made contact with the developing macrogamete (Figs. 14, 15).

Oocysts harbored in the epithelial cells of the villi had very lightly-stained vacuolated cytoplasm surrounded by a single-layered oocyst wall (Fig. 16). The nucleolus of the oocyst was not as prominent as that of the macrogamete. Eleven oocysts were 13.6-19.8 X 7.4-12.5 μ (mean, 16 X 9.9 μ). The oocysts in tissue were approximately 40% smaller than the average size of oocysts discharged in feces. This smaller size was due to shrinkage to the tissue during preparation of the slides.

Fig. 1. Sporulated oocyst.

Fig. 2. Excysted sporozoite.

Fig. 3. Young first generation schizont.

Fig. 4. Mature first generation schizont.

Fig. 5. First generation merozoite.

Fig. 6. Young second generation schizont.

Fig. 7. Mature second generation schizont.

Fig. 8. Young microgametocyte.

Fig. 9. Intermediate microgametocyte.

Fig. 10. Nearly mature microgametocyte.

Fig. 11. Mature microgametocyte with microgametes.

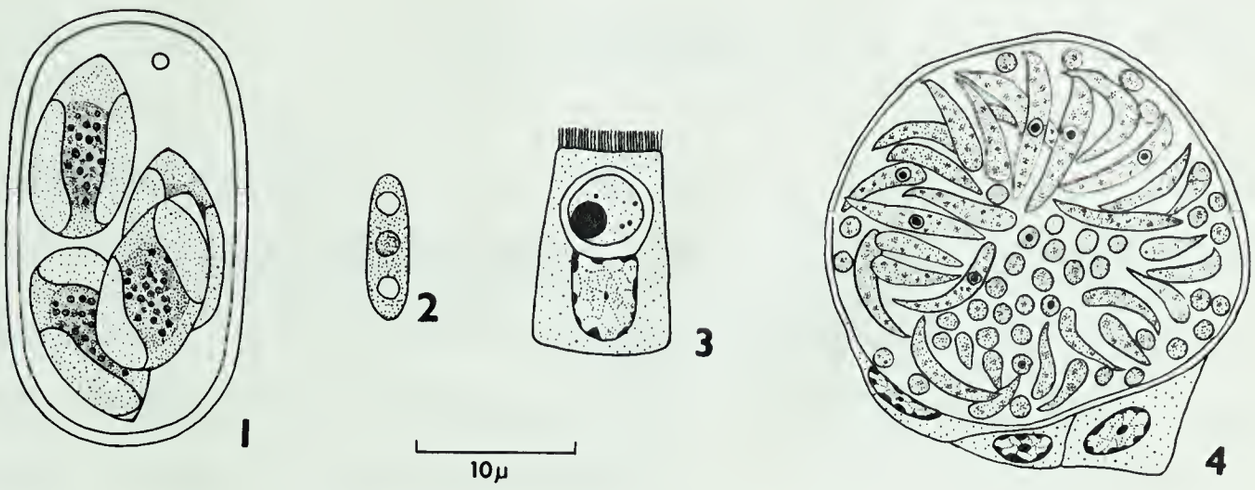
Fig. 12. Young macrogamete.

Fig. 13. Young macrogamete.

Fig. 14. Intermediate macrogamete.

Fig. 15. Nearly mature macrogamete.

Fig. 16. Oocyst in tissue.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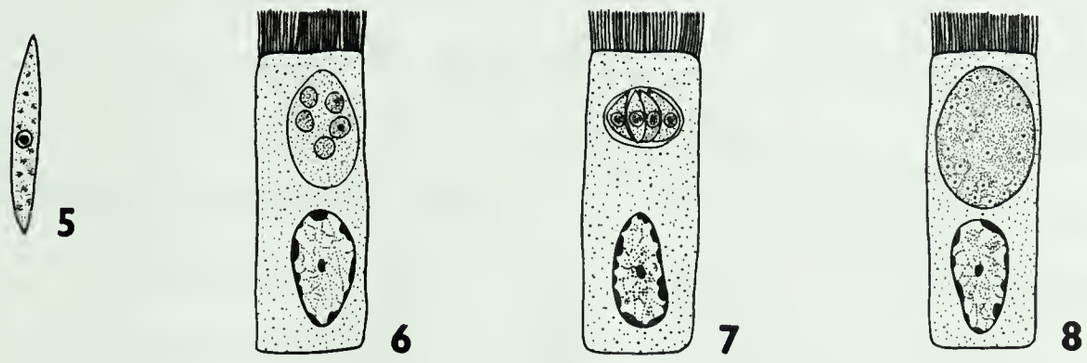
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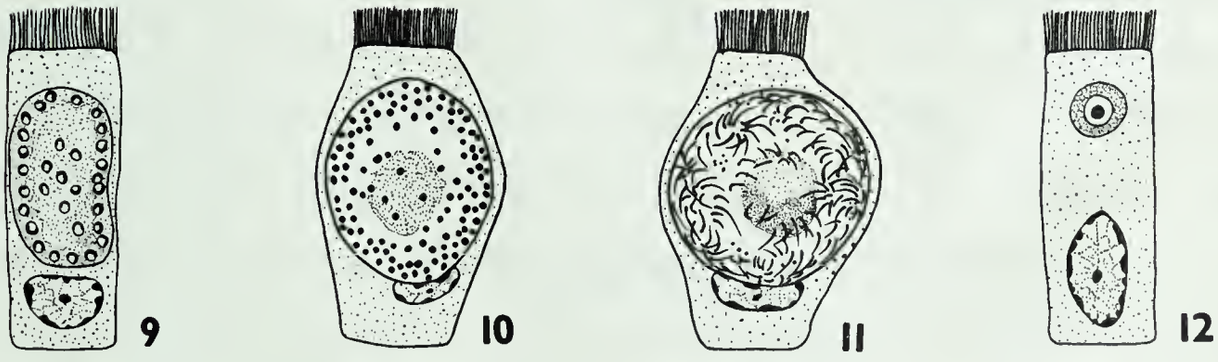
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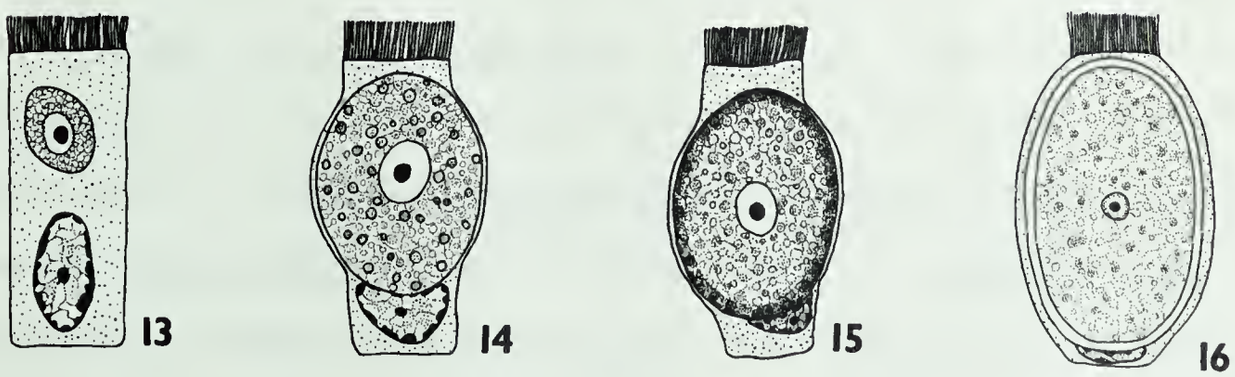
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IV DISCUSSION

1. Ectoparasites

Tamiasciurus hudsonicus is the natural host for five species of fleas; namely, *Megarthroglossus divisus exsecatus*, *Monopsyllus ciliatus protinus*, *M. vison*, *Orchopeas caedens* and *Tarsopsylla coloradensis*. In addition 12 other species have also been reported in red squirrels (Holland, 1949). Only two species, *Monopsyllus vison* and *Orchopeas caedens*, were found on red squirrels from the Gorge Creek area. These two species are common fleas whose distribution appears to be governed by the occurrence of red squirrels. Both species have been reported previously in Alberta. It is interesting to note that *Tarsopsylla coloradensis* was not found in any of the squirrels from Gorge Creek whereas this species was found by Holmes (unpublished) on some of the squirrels he examined from Gorge Creek in 1960-63. This can be explained since *T. coloradensis* is primarily a nest flea (Holland, 1949), and the possibility of finding it on a squirrel outside its nest is small.

Hoplopleura sciuricola was the only species of lice found on red squirrels from Gorge Creek. The same species was found on *Sciurus carolinensis* in Minnesota (Green, 1938) and *T. hudsonicus* in Alaska (Gill, 1954). *Neohaematopinus sciurinus*, *Enderleinellus nitzchi* and *Enderleinellus tamia-*

sciuri were also reported to infect red squirrels (Green, 1938; Ferris, 1919-35; Kim, 1966). Ixodes sp. was the only tick found in red squirrels from the Gorge Creek area. Ixodes hexagonous had been reported (Harkema, 1936) to infect Sciurus carolinensis carolinensis. No comparison can be made for mites as they have not been identified.

2. Endoparasites

A comparison of helminth infection of squirrels obtained from Gorge Creek and Rochester showed that the percentage infection of squirrels in Gorge Creek (41 of 81,51%) is about three times higher than that of Rochester (15 of 82, 18%). In the Gorge Creek area the largest sample of red squirrels was obtained in July (32 red squirrels), when a high percentage of the squirrels were infected with helminths (66%, 21 of 32), while in Rochester, the largest sample of squirrels was obtained in April (31 red squirrels), when only one squirrel (3%) was infected. This might contribute to the three times difference in prevalence of helminths in Gorge Creek. The differences in the natural habitats of these two areas might also account for the higher prevalence of helminths in the Gorge Creek area. However, a thorough study of the ecological relationships of these two areas is needed. The rate of cestode infection was also shown to increase in squirrels of the Gorge Creek area during the months of July and August.

Rausch and Schiller (1949), in their studies on Andrya macrocephalus in vole, found that the peak of parasitism of cestodes in voles is August. The cestode population in voles was lowest or completely eliminated during the winter months. A similar situation might exist for Andrya primordialis in red squirrels. Confirmation of the latter point is not possible as no examination of squirrels was done during the winter months. The Rochester data also indicated a rise in cestode population in August. The few squirrels obtained in July might account for the low infectivity during this month.

The high percentage of Taenia rileyi larvae found in red squirrels suggested that they might play an important role as an intermediate host of T. rileyi. Adult T. rileyi is known to infect lynx (Van Zyll de Jong, 1966). In contrast T. mustelae is known to infect members of the genus, Mustela (Yamaguti, 1959); adults had been found in the long-tailed weasel (Mustela frenata) in the Gorge Creek area (Holmes, unpublished). There is no previous record of cysticercus of Cladotaenia sp. in the red squirrel. Cladotaenia spp. are the parasites of Falconiformis (Yamaguti, 1959). Plerocercoids of Paruterina candelabraria have not been reported previously in red squirrels. Freeman (1957) found adult P. candelabraria in the snowy owl (Nyctea scandiaca). His experimental administration of P. candelabraria eggs to T. hudsonicus failed to produce plerocercoids. However, similar treatments produced plerocercoids in the mesenteric lymph nodes and liver of other rodents (Tamias striatus,

Peromyscus maniculatus, Clethrionomys gapperi and Mus musculus).

Citellinema bifurcatum is the most common nematode found in sciurids. This nematode is known to infect Glaucomys sabrinus macrotis, Marmota monax, Tamias striatus, Sciurus niger rufiventer and Sciurus carolinensis (Rausch and Tiner, 1948), indicating its lack of host specificity. They had been reported over an area extending from Colorado, Wyoming, Saskachewan and Manitoba eastward to Maine. More recently, it had been reported in red squirrels from Maryland (Lichtenfels and Haley, 1968).

Although Syphacia thompsoni had been reported from red squirrels (Rausch and Tiner, 1949; Lichtenfels and Haley, 1968), the Syphacia sp. found in this study was not S. thompsoni since they were much larger than S. thompsoni. Rausch and Tiner (1949) only found one of 59 red squirrels infected with S. thompsoni. It is interesting to note that although gravid pin worms (Syphacia sp.) were found in my red squirrels, eggs were not detected in the feces. It is possible that the gravid worms were shed and whole worms were eaten by new hosts to acquire infection as suggested by Noble (1966). In one case I found a partially digested Syphacia sp. in the stomach of a red squirrel, which is evidence to support the above hypothesis.

Physaloptera sp. had been reported in Sciurus carolinensis leucotis, Citellus franklini, and Citellus t. tridecemlineatus (Rausch and Tiner, 1948). Physaloptera massino has

been reported (Morgan, 1943) from Sciurus niger and Citellus t. tridecemlineatus in Wisconsin and Minnesota. McLeod (1933) reported P. spinicauda from Citellus sp. in Manitoba. Immature Ascaris sp. was also reported in T. hudsonicus (Rausch and Tiner, 1948). However their occurrence was rare and this is in agreement with the present study.

Herman and Price (1955) used a concentration technique and found that all 97 grey squirrels (Sciurus carolinensis) they examined were infected with Hepatozoon; however only 50% of the 160 grey squirrels were infected when stained smears were examined. Using the latter technique only 28% of the 63 red squirrels I examined were infected with Hepatozoon. Since the concentration technique could not be used in this study the prevalence of Hepatozoon infection was undoubtedly higher than what I was able to find by examining only blood smears. In contrast to the findings of Herman and Price where the gametocytes were found mainly in white blood cells, the parasites in this study were found only free in the plasma.

Morphologically the oocysts of Eimeria toddi described by Dorney (1962) and those found in red squirrels in Alberta were similar. Fifty-one sporulated oocysts of E. toddi in Wisconsin were 36-45 X 27-36 μ, with a mean of 40 X 32 μ (Dorney, 1962). Fifty unsporulated oocysts of E. toddi in red squirrels from Alberta had a greater range in size (29-46 X 24.5-33.7 μ; mean, 36 X 29 μ). Soon and Dorney (1969) measured 294 E. tamiasciuri oocysts from red squirrels in Ontario and

reported the following size range: 16.6-33.3 X 10.6-18.6 μ, with a mean of 27 X 15 μ. Thus the size of oocyst of E. tamiasciuri from red squirrels in Alberta and Ontario was similar.

Despite different geographic locations the prevalence of E. tamiasciuri and E. toddi are quite similar in Wisconsin and Alberta. Dorney (1962) found E. tamiasciuri in 98% of 50 T. hudsonicus and E. toddi in 6% of 36 T. hudsonicus in Wisconsin. These prevalence levels are nearly identical with those obtained for the coccidia found in red squirrels in Alberta (E. tamiasciuri, 97% of 171; E. toddi, 6% of 171). Soon and Dorney (1969) found E. tamiasciuri in red squirrels in Ontario, but they did not find E. toddi. My study is the first to report the occurrence of E. toddi in Canada. Dorney (1968) suggested that the great difference in prevalence of E. tamiasciuri to E. toddi might be due to evolution of these two species from different phylogenetic lines. Oocysts of E. tamiasciuri have thin smooth walls and sporulate rapidly within two to three days. In contrast, E. toddi oocysts have thick rough walls, and require at least one week to sporulate. Consequently, E. tamiasciuri is more likely to infect its host than E. toddi. Also the productivity of the two species differs considerably. Oocyst counts from red squirrels collected over a three year period showed a ratio of 26 E. tamiasciuri oocysts to one E. toddi oocyst (Dorney, 1966). Life cycle studies on E. toddi are needed before sound comparisons can be drawn between E. tamiasciuri and E. toddi.

The prepatent period of 5½ days for E. tamiasciuri is tentative because only one red squirrel was used. Continuous reinfection made it impossible to determine the patent period under the conditions in which the animals were housed.

The life cycle of E. tamiasciuri can be postulated based upon the stages found. The sporozoites excyst from the oocysts in the small intestine and invade epithelial cells of the crypt of Lieberkühn. Within 24 hours the trophozoite develops into a young schizont and it takes about three days to develop into a mature first generation schizont, containing 30 - 60 merozoites. The merozoites released from the first generation schizonts invade the epithelial cells of the villi, where they develop into second generation schizonts. The mature second generation schizont is much smaller in size and contains four to 16 merozoites. Developing gametocytes and oocysts were always found in the epithelial cells of the villi along with mature second generation schizonts. The site of infection of schizont, and the simultaneous appearance of schizont and gametocytes suggest that the gametocytes develop from this generation of schizonts. Second generation schizonts and the gametocytes develop and reach maturity rapidly when compared with the time required for the first generation schizont development.

The excysted sporozoites of E. tamiasciuri have one refractile body at each end. They are spherical and about the same size. However, Todd and Hammond (1968) found that the sporozoite of E. callospermophili has a very big posterior refractile body, which occupied half the length of the

sporozoite. The anterior refractile body is small and spherical. Similar large posterior refractile bodies were observed by Todd and Hammond (1968a) in the sporozoites of E. larimerensis and by Todd, Hammond and Anderson (1968) in the sporozoites of E. bilamelata. E. callospermophili, E. larimerensis and E. bilamellata are all coccidia of ground squirrels.

These three species of coccidia in the ground squirrel are found in the epithelial cells of the jejunum and the ileum, never in the duodenum and colon. In cases of heavy infection, gametocytes were also found in the cecum. In the red squirrel, E. tamiasciuri infects the epithelial cells of the entire small intestine. Prasad (1960) and Webster (1960) found that E. neosciuri in grey squirrels infected the epithelial cells of the ileum.

The orientation of the coccidium parasite in the host cells also differs from species to species. Eimeria tamiasciuri, E. neosciuri, E. callospermophili and E. bilamellata are all situated at the distal end of the host cell, above the nucleus, whereas E. larimerensis is situated below the host cell nucleus.

In the endogenous studies of the three species of ground squirrel coccidia, the trophozoites have no refractile body. However, the spherical young schizont of E. tamiasciuri has a prominent, darkly-stained refractile body situated eccentrically in the parasite. Chobotar, Hammond and Miner (1969) made similar observations in E. auburnensis in calves. Fayer

and Hammond (1967), in their study of the development of first generation schizonts in tissue culture, showed that as the sporozoite transformed into the trophozoite the ellipsoidal posterior refractile body became spherical and eccentrically situated in the trophozoite. In a more recent paper, Fayer and Hammond (1969) were able to show that the anterior refractile body of E. bovis sporozoite migrated posteriorly to combine with the posterior refractile body. The trophozoites of E. neosciuri in the grey squirrels described by Prasad (1960) and Webster (1960) have no refractile body.

The two asexual generations of E. callospermophili and E. larimerensis were all found in the epithelial cells of the villi. The second generation schizonts of both species are larger and produce more merozoites than the first generation schizonts. In contrast to the above, the first generation schizonts of E. tamiasciuri are much bigger and produce more merozoites than the second generation schizonts. The smaller schizont is considered to be the second generation schizont because of its simultaneous appearance with the gametocyte and its same site of infection as the gametocyte. In their study of E. callospermophili and E. larimerensis, Todd and Hammond (1968, 1968a) found mature second generation schizonts appeared simultaneously with the gametocytes.

Several stages of microgametogony were observed. Two of them are of special interest, namely, the stage with nuclei undergoing nuclear division and the one with chromatin aggregating along the border at one side of the nucleus,

giving the appearance of a C-shape. These stages were not described from the ground squirrel coccidia (Todd and Hammond, 1968, 1968a). However, Lapage (1940), in his study of the guinea pig coccidium, E. caviae, showed photomicrographs of microgametocytes which were undergoing nuclear division and microgametocytes with chromation aggregating along one border of the nucleus before each nucleus formed the body of a microgamete. This later stage is identical with the one with C-shaped nuclei described for E. tamiasciuri (Fig. 9). The photomicrographs also included microgametocytes with nuclei, which appeared as dark bodies, arranged near the surface. Each nucleus represented a young microgamete. This stage corresponds to what was observed in one of the stages described for E. tamiasciuri (Fig. 10). Subsequently, the young microgametes elongated and transformed into mature microgametes.

Two types of granules were seen in the macrogamete. The granules that were more deeply stained migrated to the periphery of the gametocyte. The other type was eosinophilic and remained in the cytoplasm. Both types of granules probably contribute towards the formation of the oocyst wall. Scholtyseck, Hammond and Ernst (1966), in their study of fine structure of the macrogametes of E. stiedae, E. bovis, E. auburnensis and E. perforans found two types of granules, the wall-forming bodies and the dark bodies. The dark bodies were always situated at the periphery, and the authors suggested that the dark bodies are the plastic granules fre-

quently observed under the light microscope. The wall-forming bodies were always dispersed deeper in the cytoplasm. Scholtyseck and Voigt (1964) found two types of wall-forming bodies as well as dark bodies in their study of E. perforans. Thus the eosinophilic granules observed in E. tamiasciuri may correspond to the wall-forming body. The exact role of these two types of granules in oocyst wall formation is still unclear and requires further study.

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VI APPENDIX

Measurements of 69 Tamiasciurus hudsonicus from Gorge Creek, 1968-69.

	Length in mm. Body	Tail	Hind foot	Body wt. in gm.	Sex
May 1968	268	80	45	200	♀
	300	124	47	190	♀
	350	131	51	225	♂
	321	118	51	240	♂
	338	134	46	210	♂
	326	119	49	235	♂
	318	109	49	230	♂
June 1968	308	119	49	235	♀*
	320	123	48	225	♀*
	308	104	47	235	♀
	357	110	54	220	♂
	318	128	49	230	♂
	347	133	54	250	♂
	329	130	50	245	♂
	321	124	52	210	♂
	334	132	51	235	♂
	326	114	53	190	♂
	335	129	49	240	♂
	308	105	51	260	♂
	309	117	48	220	♂
	321	125	49	275	♂
	272	86	46	200	♂
July 1968	333	130	51	260	♀
	332	134	53	275	♀*
	330	130	49	230	♀*
	330	122	52	295	♀
	328	125	51	220	♂
	300	117	46	210	♂
	322	126	50	235	♂
	343	131	52	240	♂
	316	122	50	250	♂
	324	122	50	240	♂
	322	123	54	255	♂
	323	122	52	240	♂
	301	119	50	195	♂
	312	116	48	240	♂
	322	126	52	245	♂
	309	114	47	220	♂
	326	130	51	245	♂

	Length in mm. Body	Tail	Hind foot	Body wt. in gm.	Sex
July 1968	330	124	52	260	♂
	295	111	48	215	♂
	328	130	53	255	♂
	321	122	50	245	♂
	318	122	51	225	♂
August 1968	308	120	48	220	♀
	315	124	47	240	♀
	303	120	45	245	♀
	318	130	45	195	♀
	323	115	50	235	♂
	328	129	51	270	♂
	327	127	51	225	♂
	285	81	48	255	♂
May 1969	335	136	50	225	♀*
	300	110	49	215	♀
	325	120	51	235	♀
	310	120	50	235	♀
	325	125	49	220	♀
	328	125	48	240	♀
	318	116	50	240	♀
	346	135	46	225	♂
	350	131	48	225	♂
	320	127	50	220	♂
	330	130	48	245	♂
	330	129	48	200	♂
	320	123	51	175	♂
	295	112	50	200	♂
	300	110	50	225	♂
	310	110	48	225	♂
	346	128	49	270	♂

* Pregnant